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Mast cells in tissue response to dentistry materials: an adhesive resin, a calcium hydroxide and a glass ionomer cement

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Abstract

Synthetic materials used in dentistry may trigger various inflammatory responses. In order to evaluate biocompatibility, standardized implants of Calcium Hydroxide (CH), Glass Ionomer Cement (GIC) and Light-activated Dental Adhesive (LDA) were surgically introduced into Wistar rats' back bone. Six (experimental) animal groups, five each, and two Sham (S) groups were studied after 15 and 30 days from surgery. In each animal, the density of mast cells and interstitial fibrosis volume was evaluated by quantitative light microscopy. In addition, the interaction between the disk material and its fibrous capsule was evaluated by scanning electron microscopy. The density of mast cells per area (N_A[mast cells]) was lower in CH group than in LDA group. GIC group displayed N_A[mast cells] results intermediate between CH and LDA groups ($p<0.05$). The smallest interstitial fibrosis volume density (Vv[f]) was observed in CH group, then in GIC group, while the greatest in LDA group. After 30 days, the fibrosis in LDA group was 30% higher than in CH group ($p<0.05$). In S group, discreet fibrosis restricted to surgical area was present, with few mast cells near the vessels. Significant interaction between fibrous capsule and the surrounding disk material was most evident in CH group. The implanted materials induced mast cell migration, distinct fibrosis development, suggesting that CH is the most biocompatible material among those tested.

Keywords: biocompatibility - mast cell - fibrosis - calcium hydroxide cement - glass ionomer cement - dentin adhesive

Introduction

Mast cells (MCs) participate actively in wound healing, a continuous process that starts after injury and has several phases: inflammation, tissue granulation and matrix formation, and remodeling. MCs participate in all the stages of wound healing, acting on vascular permeability angiogenesis and collagen formation [1]. In artificially wounded skin of rats, the number of these cells increases in the fibrous areas, reaching normal values at day 10 [2]. In normal human skin wound healing, the number of MCs was unaltered during the first 2 days of healing, but thereafter it steadfastly increased

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between days 4 and 10. Afterward, its density slowly decreased and finally (day 21) reached a baseline level similar to that observed between days 0 and 4 [3].

It is now clear that mast cells can participate in persistent (and even chronic) inflammatory, immunologic and fibrotic responses [4, 5]. MCs can affect the fibroblast functional behavior and, consequently, the fibrosis process, by releasing pre-formed mediators – such as histamine, proteoglycans, proteolytic enzymes –, newly formed mediators and cytokines [6]. MCs can be related to excessive deposition of extracellular matrix, as in hypertrophic scars and in ischemic segments of myocardium [7, 8].

A short time ago, it has been shown that MCs regulate fibroblast proliferation by heterotypic cell-cell contact and contribute significantly to wound repair. Co-culture of dermal fibroblasts with a human mast cell lineage significantly intensified the contraction of the three-dimensional collagen lattices. MCs alone failed to contract the gel, and the histological analysis demonstrated that, within the lattices, mast cells were localized in close contact to (or attached to) fibroblasts [9, 10], regulating proliferation by cell to cell contact [3, 11].

The presence and disposition of fibrous tissue [12, 13], around implants of dental materials, are indicative to tissue response [14]. So, the biocompatibility of a material is inversely related to the amount of fibrosis developed around it.

Materials and methods

Eight groups of five male Wistar rats each were studied (three experimental groups and one *sham* group for each time setting). The experimental groups were: the light-cured dentin adhesive group (LDA), the calcium hydroxide group (CH) and the glass ionomer cement group (GIC). The experimental and *sham* groups were examined at two time settings, 15 and 30 days after implant.

In the beginning of the study, animals had a body mass of 237.4±17.4g (mean±SD). All animals had free access to water and food (Nuvilab®, Rio de Janeiro, Brazil). This study was approved by the State University of Rio de Janeiro Standing Committee on Animal Research. The investigation conforms to the "Guide for the Care Use of laboratory Animals" published by the US National Institute of Health (NIH Publication No.85-23, revised 1985).

A standardized disk (4 mm diameter and 1 mm thick) of the respective material was superficially implanted in the backbone of each animal under anesthesia. The disks were prepared as follows: the LDA disks (Single Bond/3M, Dental Products, St. Paul, MN, USA) were polymerized at 0.5 cm for 40 sec (Translux CL, Heraeus Kulzer Wehrheim, Germany). The radiometer Model 100 (Demetron, Division of Kerr Corp., Danbury, CT, USA) was employed to check the light intensity before each procedure. The light-curing unit did not emit light intensity lower than 500 mW/cm^2. The CH disks (Dentsply, Brazil) were obtained mixing 1.6 g of calcium hydroxide with 1 ml of 70° ethanol. The GIC disks (Ketac-Bond/ESPE, Seefeld, Germany) were obtained following manufacturer's instructions.

At the end of the experiment (the 15^{th} and 30^{th} days, respectively) animals were deeply anesthetized (Thiopental 15 $mg \cdot kg^{-1}$ intraperitonealy) and then sacrificed (exsanguination). The implants and adjacent tissue were removed; a similar tissue specimen (topography and size) was removed from the *sham* group. Fragments were placed in fixative (freshly prepared 4% w/v formaldehyde in 0.1M phosphate buffer pH 7.2) for 48 hours at room temperature, embedded in Paraplast plus® (Sigma, St Louis, USA) and sectioned at 5μm thick and mounted. Sections were stained with picro-sirius red and toluidine blue.

The quantitative light microscopy used a video microscopic system (Leica DMRBE microscope and a Kappa video camera), as previously described [17]. The volume density of fibrosis was estimated using the point-counting method with the M42 test-system [18] (P_T is the total number of test-points and Pp is the relative points hitting the fibrotic area). The numerical density of the mast cells (N_A[mast cell]) was also determined into a 70,400μm² frame. The data were counted in 15 different microscopic fields per animal (75 fields per group) in random sections [19-21].

For scanning electron microscopy (SEM), the material was washed in current water by 12 hours for removal of the fixative agent. Then, the implants and the adjacent tissue were cleft to analyze the disk-fibrous capsule interface.

The material was observed under a SEM LEO 1450VP under temperature -25ºC, pressure 100Pa, voltage acceleration 15KV and detector VPSE (Variable Pressure Secondary Electron). During the observation, the selected images were stored.

The differences among groups were assessed by the Kruskall-Wallis non parametric analysis of variance and the Kolmogorov-Smirnov test using a significance level of 0.05 [22].

Results

In extracellular matrix, LDA and GIC groups had higher MCs number than S and CH groups. The aspect of the subcutaneous tissue showing fibrous areas in different groups varied from little (S group) to intense fibrosis (LDA, CH and GIC groups) (Fig. 1).

The number of MCs in the different groups is shown on Fig. 2. There were more MCs in LDA, CH and GIC groups than in S group. However, the N_A[mast cell] showed no difference between the S and the CH groups in both experimental time settings ($p>0.05$). The LDA group showed an increase of 200% in N_A[mast cell] when compared with the CH group, and more than 400%, when compared with the S group ($p<0.05$). The N_A[mast cells] of was more than 400% greater in the GIC group after 15 days vs S group ($p<0.05$). There was no statistical variance for N_A[mast cells] in time.

The results of subcutaneous fibrosis are shown in Fig. 3. All implants induced significant fibrosis (increase of Vv[f]) versus sham operated. At both time settings, the greatest Vv[f] was found in the LDA group (more than 1,000% versus S). After 30 days, the Vv[f] was 50% greater in the LDA group than in the CH group.

SEM micrographs showing the disk-fibrous capsule interface are displayed in Fig. 4. The main differences were observed between the CH and the other groups. The LDA and GIC groups presented dense fibrous capsules totally separated from the disk, with organized fibers, parallel to the disk, for the GIC group. The CH group exhibited a slack capsule with an uniform layer of conjunctive tissue at the disk-fibrous capsule interface.

Discussion

In order to assess the biocompatibility of the materials used in this study, we have determined by stereology the N_A[mast cell] and the Vv[f] adjacent to these materials. Stereology provides a much more informative measurement than the thickness, as it determines three-dimensional quantitative parameters of morphological structures from bi-dimensional counting, using geometry and probabilistic statistics [15–16].

In all experimental groups there was an increased number of mast cells when compared to S group. However, the smallest density of these cells was present in CH experimental group. N_A[mast cells] in GIC group was at least four times that of S group, after fifteen days. There was no significant difference in N_A[mast cells] in the two time settings, despite the N_A[mast cells] decreased in all groups between 15 and 30 days.

Examining standard deviations, one may observe greater values in CH than in LDA group which suggests an irregular mast cells distribution, more concentrated around vessels in CH group comparing to a more uniform distribution in the fibrotic region, in LDA group.

CH produced a less dense fibrous capsule than the other experimental materials. In LDA and GIC groups, there was a denser fibrosis, as well as a greater number of mast cells, at day 15. It is to notice that LDA group constantly displayed the biggest Vv[f] among all experimental groups.

LDA had a considerable increase in Vv[f] from day 15 to day 30, without reaching statistical significance.

Earlier studies assessed the biocompatibility of endodontic materials based on CH [12-13, 23-24]; CH cements [14]; GIC [25-26] and dentin adhesive systems [14, 27] measuring the inflammatory infiltrate and the thickness of the fibrous capsule surrounding material samples implanted in the subcutaneous tissue of rats. Intensity and length of responses vary among the endodontic materials as a result of the different anti-septic agents present in their formulas. A moderate inflammatory reaction (mononuclear cells and formation of a thin, fibrous capsule) after short (5 to 7 days) exposure to GIC and CH cements, was noted. Near the 30th day, there is a rearrangement of the fibrous capsule around the implants with appearance of macrophages and giant cells. Obvious repair and a thin, fibrous layer surrounding the implants was observed using CH cement. Some of these studies examined the change of the inflammatory infiltrate

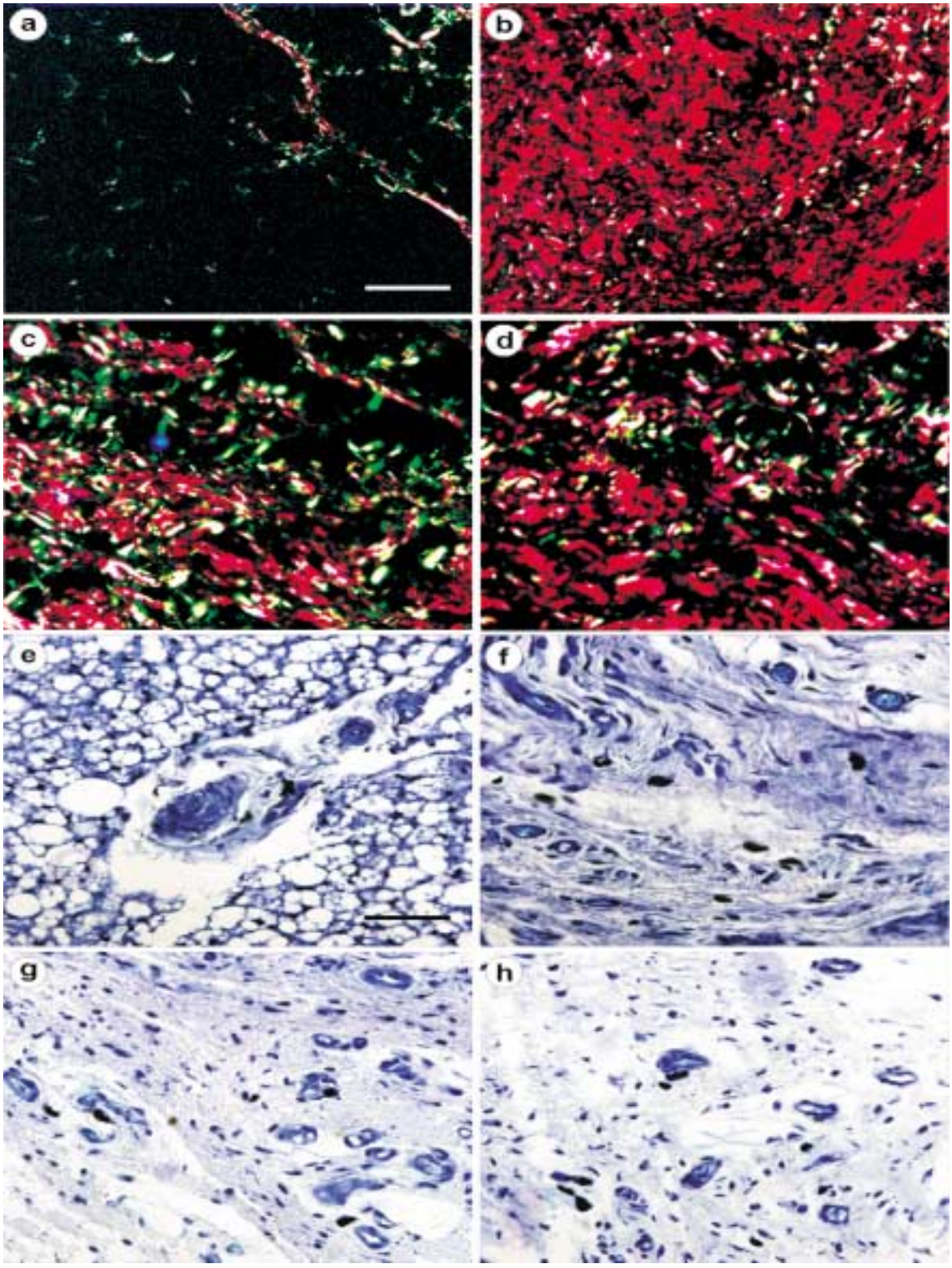

Fig. 1 Photomicrographs of the subcutaneous tissue sections (30 days) stained with picro Sirius red (a-d) or toluidin blue (e-h). Collagen distribution, observed under polarization, is represented by scarce green-yellow fibers in *sham* group (a), by a large amount of red-yellow fibers in LDA group (b), and mainly yellow collagen fibers in CH (c) and GIC (d) groups. Mast cells are rare in *sham* group (e), in LDA there is a large amount of mast cells (f), and in CH (g) and GIC (h) groups a moderate amount of mast cells is observed (Bar = 50μm).

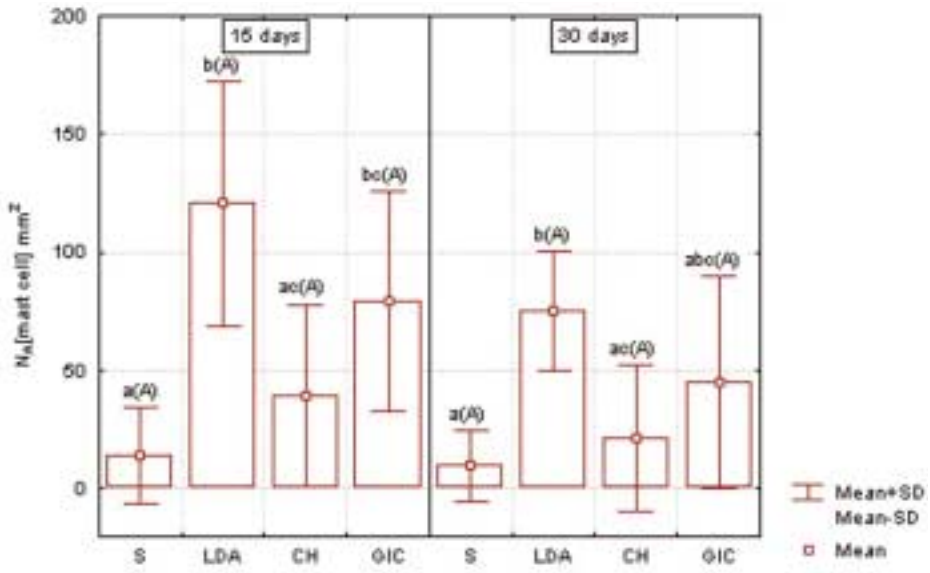

Fig. 2 Numerical density of mast cells per area (N_A[mast cells]) in the subcutaneous tissue of rats at days 15 and 30. Groups: Sham (*S*), Light-cured Dentin Adhesive (LDA), Calcium Hydroxide (CH) and Glass Ionomer Cement (GIC). Different alphabetical symbols on the bars mark significant difference ($p<0.05$).

in fibrous tissue [27], measuring the thickness of dentin adhesive systems and CH cement around the implants [14]. In the present study, such inflammatory infiltrate was not detected, probably due to the fact that the first evaluation moment was at 15 days, long enough for inflammatory process to finish, allowing initiation of the late repair phase [2].

In our study, CH group provided appropriate repair in 15 days of experimentation, producing a less dense fibrosis (possibly due to the smaller mast cells migration) and hence a slighter fibroblast-mast cell interaction. From days 15 to 30, Vv[f] kept stable and N_A[mast cells] diminished at half.

The largest mast cells migration occurred in LDA group, with high fibroblast-mast cell interaction and development of a denser capsule at 15 days. Between days 15 and 30, Vv[f] increased and N_A[mast cells] decreased at two thirds.

After fifteen days GIC group presented a bigger fibrosis, as well as mast cells migration, than CH

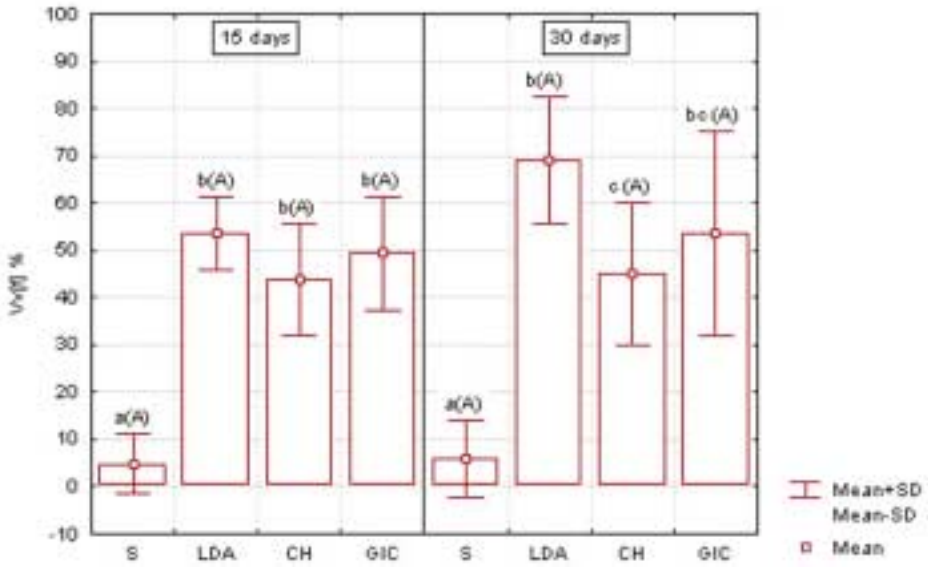

Fig. 3 Volume density of fibrosis (Vv[f]) in the subcutaneous tissue at days 15 and 30. Groups: *Sham* (*S*), Light-cured Dentin Adhesive (LDA), Calcium Hydroxide (CH) and Glass Ionomer Cement (GIC). Different alphabetical symbols on the bars mark significant difference ($p<0.05$).

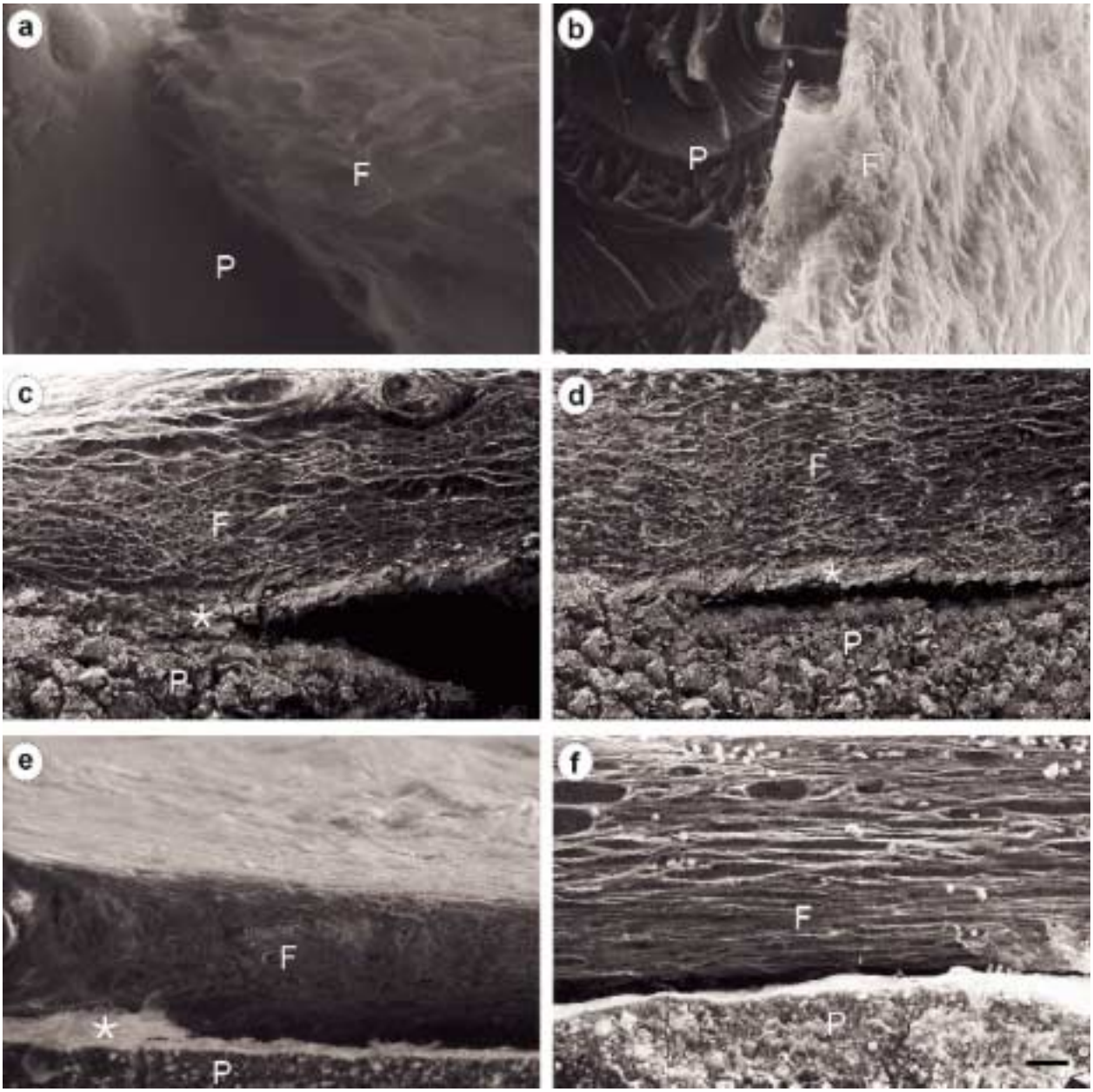

Fig. 4 Scanning electron micrographs showing the disks (**D**) and the fibrous capsules (**F**) that surround them 15 days (**a**, **c, e**) or 30 days (**b**, **d**, **f**) after implantation of the disk in the animal's subcutaneous tissue. On **a** and **b** (LDA group) we can observe a dense fibrotic capsule totally detached from the disk. On **c** and **d** (CH group) we can observe the capsule floppily organized, and intimate interaction (*****) between connective tissue and CH group. On **e** and **f** (GIC group) a denser fibrous capsule only in the small interaction region (*****) is showed between the capsule and GIC's disk. On **f** the capsule is more organized, with bundles parallel to the disk (Bar = 20μm).

group, which suggests an active fibroblast-mast cell interaction. From day 15 to 30, Vv[f] increased a bit and N_A[mast cells] decreased.

There was a reduction in N_A[mast cells] values in all groups at day 30. However, the values corresponding to this moment in LDA and GIC groups were double and similar with the value obtained in CH group at 15 days, respectively.

Some relevant aspects are observed under the SEM of the cloven disks and their fibrous capsules.

Contrary to LDA and GIC groups, CH group showed a loose capsule in total conjunction with the disk through a neat layer composed of collagen fibers. In LDA group, the material did not present any link with a dense network capsule and irregular disposed fibers. In GIC group, the capsule presented parallel fibers with inclusion of silica particles among their sheaves. Lack of capsule-disk interaction may show biological incompatibility of the materials.

This study documented the increase number of mast cells and the development of fibrosis in the subcutaneous tissue of rats exposed to implants of different dental materials. It was observed that the disks of the implanted materials presented fibrotic capsule after 15 days, slighlty changed at day 30. It all implies that the inflammatory process resolution, mast cells migration and fibrosis development are linked to the biocompatibility of the tested material. Besides that, the increase in number of mast cells interacting with fibroblasts allows a greater production of collagen, increasing the fibrous capsule density. So, with the formation of less dense fibrous capsule and the early demobilization of the mast cells, CH group turned out to be the most biocompatible among the groups evaluated in this study.

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